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Characterization of cyclic nitramine explosives in Wainwright firing range soil during pilot scale treatment with Fe(0) and phytoremediation.

Final Report

presented to

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May 8, 2000

Canada

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
08 MAY 2000		**00-00-2000 to 00-00-2000**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Characterization of cyclic nitramine explosives in Wainwright firing range soil during pilot scale treatment with Fe(O) and phytoremediation	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Emiromental and Analytical Chemistry Group,CNRC-Biotechnology Research Institute,6100 Royalmount Avenue,Montreal, PQ H4P 2R2,**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

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15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**28**	

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ABSTRACT

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INTRODUCTION

A number of testing and training sites have been identified by the Canadian military as possible sources of soil and groundwater contamination by various formulations of the explosives RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine), HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine) and TNT (2,4,6-trinitrotoluene). In some cases the level of contamination was observed to be less than 10 mg/kg for any of the three explosives, whereas at other anti-tank firing ranges the HMX contamination ranged from 1,640 mg/kg near one target to 2.1 mg/kg at a distance 15 m away from the target (Jenkins *et al* 1998; Thiboutot *et al* 1998). A number of methodologies (non-valent iron reduction, composting, phytoremediation) are currently under consideration for the rehabilitation of these lands.

This report provides quantitative data in support of a pilot greenhouse study that examined the potential of phytoremediation and non-valent iron addition as applied to the climactic and soil conditions at an anti-tank firing range in Wainwright Alberta. The data is largely presented as HMX dry weight concentrations for selected treatments of the Wainwright soil and for the shoot and root tissues of the five plant species selected for testing. While the determination of the explosive content in soil is a widely recognized standard protocol (EPA 8330), no recognized standard method currently exists for the analysis of explosives and their degradation products in plant tissues. Most of the protocols in current use are variations of EPA 8330 constructed to minimize additional equipment requirements. The selected protocol for plant tissue (Larson *et al*, 1998) serves well for the comparison of HMX content with soil samples, but the various steps (homogenization, lyophilization, silica chromatographic cleanup) were performed with method development in mind to improve general efficiency and the recovery of volatile cyclic nitramine degradation products.

The data will serve to resolve the mode of remediation (ie reductive elimination by iron, phytoextraction, phytodegradation), the selection of plant species, and the projected time period for successful application of these strategies.

EXPERIMENTAL

Soil samples

The pilot study made use of soil with the following amendments: contaminated soil only (treatment 1), clean soil with plants (treatment 2), contaminated soil with plants (treatment 3), contaminated soil with manure (treatment 4), contaminated soil with iron and manure (treatment 5), contaminated soil with iron, manure and plants (treatment 6), clean soil with manure (treatment 7), clean soil with manure and iron (treatment 8). All samples were shipped frozen in polyethylene jars or bags and stored at 4 °C. Soil samples were prepared and extracted using EPA 8330 as specified for the ppb analysis of certain explosives in soil and sediment matrix. The following modifications apply:

- **Sample preparation**: Preliminary and mid-point samples were lyophilized in place of air drying to constant weight for the removal of moisture. The samples were then passed through a 32 mesh sieve before extraction. In the case of final samples, the entire provided sample (400 g) was spread in a Pyrex dish, mixed with acetone to a paste, and then air dried for 24 to 48 hours before sieving to reduce the spatial heterogeneity of crystalline HMX in the soil.

- **Extraction:** Preliminary samples were extracted with the addition of 2 g of sieved soil to 10 ml acetonitrile in an ultrasonic bath for 16 hours. For midpoint samples, 4 g of soil were added to 10 ml of acetonitrile to account for soil heterogeneity. Final samples were extracted using 16 g of soil added to 40 ml of acetonitrile in 60 ml bottles. The use of such large volumes reduced the sample capacity of the sonicator and a time study revealed that 6 hours sonication was sufficient to extract all available HMX from the Wainwright soil. Final soil samples were accordingly sonicated either for 16 hours overnight or for 6 hours during the day to increase sample through-put. Preliminary samples were extracted in triplicate. Mid-point samples were extracted singularly except in the case of treatments 1 and 4 which were performed in triplicate. Final samples were normally extracted in

duplicate. When the HMX concentration deviation exceeded 15 % of the mean for a given set of duplicates, a third extraction was completed.

Plant samples

The plant samples were prepared and analyzed for HMX using the methods outlined in the technical report Analysis of Explosives in Plant Tissues: Modifications to Method 8330 for Soil. (Technical Report IRRP-98-5, U.S., Army Corps of Engineers, Washington, DC). Four types of plant sample were provided for analysis: 1) Fresh cuttings containing stems, leaves, beans (bush bean) or seed cases and flowers (canola). 2) Dried (senescent) cuttings containing almost exclusively leaves. 3) Roots rinsed with water to remove most but not all soil material. 4) Beans harvested separately from the bush bean samples. All samples were shipped frozen in polyethylene bags and stored at -20 °C.

- Sample preparation: Following the recording of total sample weight, approximately 5 g of material was selected from each sample with care taken to provide equal amounts for each tissue present (ie stems, leaves, fine roots, coarse roots, etc). The sample was then finely cut into 2 mm pieces with scissors and transferred to a tared 100 ml beaker and the cut weight was recorded as the sample fresh weight using an analytical balance. Samples were stored on ice for immediate homogenization. The finely cut samples (approximately 4 g) were then suspended in 10-20 ml of ice cold deionized water (18 MΩcm resistivity) and homogenized using a Kinematica (Kriens Switzerland) Homogenizer fitted with a Brinkman Polytron PTA 20 S saw tooth generator (Brinkman Instruments, Mississauga ON) suitable for fibrous plant or animal tissues. Homogenization was initially performed at 5,000 RPM (1/4 of full scale setting) for 2 minute intervals with immersion of the beaker into ice. The samples were then homogenized in the same manner at full setting (20,000 RPM) until a frothy granular paste was obtained.

Immediately after homogenization, the samples were transferred to tared 120 ml Labconco lyophilization flasks and the sealed flask assemblies were immersed in a dry ice acetone bath for 20 minutes. The flasks were then connected to either a Flexi Dry FDX-1-84ACD (Flexi Dry Inc, Stone Ridge NY) or a Virtis Freezmobile 24 (Virtis, Gardner NY) lyophilizer. The samples were lyophilized until no further change in flask weight was observed (average time 20 h). Lyophilized samples were transferred to tared polypropylene vials and the lyophilized weights were recorded. The freeze dried samples were stored under aluminum foil at 4° C.

- **Extraction:** Approximately 0.2 g of freeze dried material was transferred to a 15 ml Kimax screw cap culture tube with the subsequent addition of 10 ml of acetonitrile. The capped tubes were then vortex mixed and placed in a Blackstone Ultrasonics Neptune Ultrasonic Generator (Blackstone Ultrasonics, Jamestown NY) cooled to 10 °C using a Lauda RM6 refrigerated circulating bath (Brinkman instruments, Mississauga ON). The sonication duration was 18 hours.

- **Sample clean up:** After sonication the Kimax tubes were centrifuged at 5,000 RPM for 15 minutes using a Fisher Centrific benchtop centrifuge (Fisher Scientific, Montreal QC). The supernatant was then decanted and allowed to settle for 20 minutes. A 2 ml aliquote of the supernatant was then mixed with an equal volume of deionized water and filtered using Millex HV 0.45 µm filter cartridges. The samples were then immediately analyzed (HPLC-UV).

Analysis

- **HPLC-UV analysis:** A Waters chromatographic system composed of a Model 600 pump, a Model 717 Plus injector, a Model 996 Photodiode-Array Detector and a Temperature Control Module was used for HPLV-UV analysis. The column was a Supelcosil LC-CN (25 cm, 4.6 mm, 5 µm) with the column temperature held at 35 °C.

The solvent system consisted of a methanol / water gradient at a flow rate of 1.5 ml / min. The initial solvent composition was 30% methanol and 70% water, which was held for 8 min. A linear gradient was then run from 30 % to 65 % methanol over 12 minutes . The solvent ratio was then returned to initial conditions over 5 min and then maintained for 5 min for a total run time of 30 min. The detector was set to scan from 200 to 325 nm with extraction of chromatograms at 254 nm. The injection volume was 50 µl. In general, this method has proven to be superior to that of EPA 8330 (C18 column with an isocratic 50 % water / methanol mobile phase) for the reduction of interferences The limits of detection and quantification for HMX with this method were respectively 0.05 ppm and 0.1 ppm.

- **LC-MS analysis:** A Micromass Plattform benchtop single quadrupole mass detector fronted by a Hewlett Packard 1100 Series HPLC system equipped with a Photodiode-Array detector was used. Samples (50 µl) from the extract were injected into a Supelcosil LC-CN column (25 cm, 4.6 mm, 5 µm) thermostated at 35 ° C. Two different methanol / water gradients were used at a flow rate of 1 ml / min. For the first HPLC method applied, initially, a linear gradient was run from 10 % to 20 % over 15 min, followed by a second linear gradient from 20% to 60 % over 5 min which was then held for 3 min. This solvent ratio was returned to the initial conditions over 2 min and held for an extra 10 min. For the second method, the initial solvent composition was 40 % methanol and 60 % water held for 8 min, then a linear gradient was run from 40 % to 65 % methanol over 12 min. This solvent ratio was changed to the initial conditions over 5 min and held for an extra 10 min. Analyte ionization was done in a negative electrospray ionization mode (ES -) producing [M-H] mass ions. The electrospray probe tip potential was set at 3.5 kV with a cone voltage of 30 V at an ion source temperature of 150 ° C. The mass range was scanned from 25 to 400 Da with a cycle time of 1.6 s and the resolution was set to 1 Da (width at half height). The limit of detection for this method was less than 4 ppb.

- **QA/AC:** Data were verified through instrumental calibration curves, blank runs, reproducibility and accuracy checks. Recoveries were verified by spiking non-contaminated soil samples with HMX and extracting them under the same conditions as for the contaminated soils.

RESULTS AND DISCUSSION

Soil Samples

Three preliminary analyses for contaminated soil were conducted prior to commencement of the greenhouse trial. The mean HMX concentration for the triplicate measure of these samples ranged from 28.8 ppm to 50.7 ppm (Table 1). Following the analysis of mid-point samples, the in-sample variation was reduced through the partial solubilization and redistribution of HMX in soil with the addition of acetone as described in the DREV report DREV-R-9721 (Table 2). A second measure taken to decrease in-sample variation was to increase the amount of soil extracted for analysis. As shown in Table 3, within practical limits, 16 g was the optimal amount of acetone treated soil for use in extraction. For the most part, only single measurements were taken for midpoint samples with triplicate sampling of treatment 1 (HMX contaminated soil) and treatment 4 (HMX contaminated soil with manure) to indicate in-sample variability. As shown in Table 4, the RSD for these samples was respectively 18.1 and 10.8 %. Typical differences observed for the analysis of final samples were 10 % (Table 5). The best estimate of HMX concentration in the final control samples (ie Table 5, treatment 1) was therefore 35 ppm with a 99% confidence interval of 9 ppm (ie $\mu = 35$ ppm, $\sigma = 6.1$ ppm, $n = 6$, $t_{.010} = 3.365$). The ultimate source of variability in these measurements is found in the crystalline nature of residual HMX and its spatial heterogenic effect on concentration . The level of variation is therefore dependent on the effectiveness of the on-site soil mixing method and on the practical extent to which increased sample number, sample size and sample homogenization can be

achieved prior to analysis. The effect of this variation should be given careful consideration before the establishment of field experiments.

As shown in Table 5, the observed differences in mean HMX concentration for all soil treatments were not significantly different from those of the controls. This is somewhat surprising for treatments 5C and 5D and the most likely cause for limited remediation is the limited contact of HMX with the iron particles at the trial soil conditions, (ie low moisture, neutral pH). The addition of non-valent iron did not appear to be the treatment of choice for the remediation of HMX in Wainwright soil.

Plant samples

The HMX content of midpoint and final plant samples are shown respectively in Tables 6 and 7. Once again, relatively high levels of sample to sample HMX concentration are observed for the various treatments, but the observed differences in average HMX concentration relative to controls indicated that HMX was significantly accumulated by all of the selected plant species. In general, the dried (senescent) shoot samples were observed to have the highest content of HMX . The fresh shoot samples were observed to have the next greatest abundance of HMX. It should be noted that in the cases of bush bean and canola the dried samples were comprised exclusively of leaves. The root samples were observed to have significantly lower HMX content. It is interesting to note that no HMX was observed in the beans collected from bush bean plants grown in HMX contaminated soil (ie treatments 3 and 6). The HPLC method had no difficulty in resolving and quantifying the HMX content of fresh shoot and senescent leaf samples (Figure 1). For root samples, the determination of HMX in alfalfa root tissue was not possible using the HPLC method as a large interference peak was observed to elute at the retention time (14.4 minutes) normally observed for HMX. The same large peak was observed in the alfalfa root extract taken from the control sample (treatment 2). LC-MS analysis of alfalfa root

samples grown in HMX contaminated soil revealed trace levels of HMX in sample extracts (approximately 0.05 mg/L, Figure 2C).

HMX metabolites and other contaminants

In the case of TNT, the covalent linking of aminonitrotoluene metabolites to complex glycols (starches) in root tissue is known and the hydrolysis of these samples in hot acid is recommended to free bound analyte. This treatment can be applied to HMX or RDX, but the formation of stable amino RDX or HMX derivatives has not been reported in the literature. No amino derivatives were observed in the LC-MS analyses of soil and plant samples. Small quantities of 1-nitroso-3,5,7-trinitro-1,3,5,7-tetrazacylooctane (MN-HMX) were observed in all of the HMX contaminated soil samples as detected by HPLC (Figure 3) and periodically verified by LC-MS (Figure 4). In addition, minute quantities (< 1 ppm) of TNT and tetryl (Figures 3, 4) were infrequently observed in the contaminated soil samples, and this observation was consistent with the explosive formulations employed on the anti-tank range. The lack of observable intermediates and the amount of HMX observed in dead leaf tissue relative to that in soil suggested that a process of phytoextraction, rather than phytodegradation was occurring. Of the species tested the HMX concentrations appeared to be greatest for rye and wheat samples. The accumulation of HMX in the leaves was a function of water transport and transpiration and the availability of HMX to the plants was limited by its low solubility (approximate maximum solubility 5 ppm in aqueous solution).

CONCLUSION

The observation of HMX in plant tissue samples for all of the 5 plant species grown in Wainwright firing range soil provided supporting evidence for the use of phytoremediation. The provision of direct evidence via HMX soil determination was hindered by variation due to the spatial heterogeneity of crystalline HMX in firing range soil. The trial did not provide conclusive supporting evidence for the use of non-valent iron as remediation technology specific to the Wainwright firing range. The high variation of HMX concentration in Wainwright soil must be accounted for in the development of further pilot or field trials.

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LIST OF TABLES

Table 1. HMX in soil samples, Preliminary Sampling Event.

Sample Prefix	Soil Sample	
	HMX[a] (mg/kg)	RSD (%)
DND-1	n.d.	n.a.
DND-2	n.d.	n.a.
DND-3	28.8	29.1
DND-4	50.7	31.4
DND-5	32.3	24.2

[a] Mean of triplicate extraction.
n.d. Not detected.
n.a. Not applicable.

Table 2. Effect of the addition of acetone during the soil preparation on the RSD value.

Description	Sample Prefix	Soil Sample	
		HMX[a] (mg/kg)	RSD (%)
Preliminary Sampling	DND-3	39.6	17.7
Mid-Point Sampling	1-4-A	29.5	13.6
	4-4	26.6	6.3
Final Sampling	1-1	31.3	40.6
	1-2	29.1	23.5
	1-3	43.9	28.8
	4-1	40.5	23.9
	4-2	20.7	10.2
	4-3	30.3	13.4

[a] Mean of triplicate extraction, 4 g of soil extracted with 10 ml acetonitrile.

Table 3. Effect of the amount of soil extracted on the RSD value and the extraction time on HMX recovery.

Amount of soil	Soil Sample DND-3	
	HMX (mg/kg)	RSD (%)
4[a]	39.6	17.7
8[a]	32.5	12.1
8[b]	31.7	17.5
16[a]	25.3	1.7
16[b]	31.8	3.3
32[a]	33.4	8.5

[a] Mean of triplicate extraction (16 hours extraction).
[b] Mean of triplicate extraction (6 hours extraction).

Table 4. HMX in soil samples, Mid-point Sampling Event.

Treatment	Sample Prefix	Soil Sample	Manure Sample
		HMX[a] (mg/kg)	HMX[a] (mg/kg)
1	1-4-A	22.4	
2	2A-4	n.d.	
	2B-4	n.d.	
	2C-4	n.d.	
	2D-4	n.d.	
	2E-4	n.d.	
3	3A-4	30.2	
	3B-4	55.6	
	3C-4	47.8	
	3D-4	51.2	
	3E-4	32.8	
4	4-4	39.1	9.1
5	5A-4	23.7	11.6
	5B-4	37.0	12.3
	5C-4	32.1	5.1
	5D-4	25.4	7.2
6	6A-4	31.0	1.3
	6B-4	19.0	10.9
	6C-4	27.5	4.7
	6D-4	87.4	6.1
	6E-4	31.9	2.3
7	7-4	n.d.	n.d.
8	8A-4	n.d.	n.d.
	8B-4	n.d.	n.d.
	8C-4	0.3	n.d.
	8D-4	2.3	n.d.

[a] One replicate was extracted, except for treatments 1 and 4 that were extracted in triplicate with respective RSD of 18.1% and 10.8%.

n.d. Not dectected

Table 5. HMX in soil samples, Final Sampling Event.

Treatment	Sample Prefix	Soil Sample	
		HMX[a] (mg/kg)	Deviation (±)
1	1-1	36.6	5.5
	1-2	30.2	3.4
	1-3	38.1	4.1
2	2A-1	traces [b]	n.a.
	2B-3	traces [b]	n.a.
	2C-1	0.5	n.a.
	2D-1	traces [b]	n.a.
	2E-1	traces [b]	n.a.
3	3A-1	37.9	4.3
	3A-2	34.7	2.9
	3A-3	28.8	0.5
	3B-1	34.7	2.1
	3B-2	41.0	1.5
	3B-3	26.9	0.2
	3C-1	34.5	1.5
	3C-2	28.6	0.1
	3C-2	28.9	0.7
	3D-1	33.6	3.4
	3D-2	30.3	1.9
	3D-3	30.9	2.6
	3E-1	27.6	1.1
	3E-2	39.5	4.2
	3E-3	35.5	2.5

[a] Mean of duplicate extraction, except for the samples from treatment 2.
[b] Below the instrumental quantification limit (0.1 ppm), but identified by LC-MS.
n.a. Not applicable.

Table 5. HMX in soil samples, Final Sampling Event, continued.

Treatment	Sample Prefix	Soil Sample		Manure Sample	
		HMX[a] (mg/kg)	Deviation (±)	HMX[a] (mg/kg)	Deviation (±)
4	4-1	31.5	0.7	15.5	0.2
	4-2	27.9	0.3		
	4-3	33.2	2.0		
5	5A-1	29.3	1.3	16.8	3.2
	5A-2	32.9	0.8		
	5A-3	30.6	1.7		
	5B-1	27.5	0.1	15.9	2.2
	5B-2	29.8	1.2		
	5B-3	27.6	0.2		
	5C-1	35.0	2.5	13.6	0.9
	5C-2	32.1	0.5		
	5C-3	38.9	5.6		
	5D-1	33.6	0.7	16.5	6.0
	5D-2	32.1	2.3		
	5D-3	24.8	1.4		
6	6A-1	32.4	3.1	2.0	0.1
	6A-2	37.2	3.7		
	6A-3	29.4	2.4		
	6B-1	27.3	1.0	3.2	0.1
	6B-2	27.7	3.9		
	6B-3	29.9	1.3		
	6C-1	34.3	3.7	4.7	0.1
	6C-2	37.8	1.9		
	6C-3	44.1	3.5		
	6D-1	28.4	1.2	4.6	0.3
	6D-2	36.2	3.4		
	6D-3	32.7	0.9		
	6E-1	41.8	4.5	5.0	1.5
	6E-2	32.1	0.1		
	6E-3	42.2	0.8		
7	7-1	traces[b]	n.a.	n.d.	n.a.
8	8-1	4.1	n.a.	n.d.	n.a.

[a] Mean of duplicate extraction, except for the samples from treatment 7 and 8.
[b] Below the instrumental quantification limit (0.1 ppm), but identified by LC-MS.
n.a. Not applicable.
n.d. Not detected.

Table 6. HMX in plant samples, Mid-point Sampling Event.

Treatment	Sample Prefix	Fresh shoots				Roots	
		HMX[a] (mg/kg)	Deviation (±)	HMX[b] (mg/kg)	Deviation (±)	HMX[a] (mg/kg)	HMX[b] (mg/kg)
2	2A-4	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.
	2B-4	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.
	2C-4	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.
	2D-4	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.
	2E-4	n.d.	n.a.	n.d.	n.a.	n.d.	n.d.
3	3A-4	121.0	3.0	18.6	0.4		
	3B-4	164.5	0.5	23.8	0		
	3C-4	197.0	n.a.	20.7	n.a.	16.4	3.15
	3D-4	60.7	10.2	16.2	2.7	18.3	4.53
	3E-4	171.0	2.0	50.6	0.6	44.9	14.3
6	6A-4	75.7	1.2	18.0	0.3		
	6B-4	115.5	1.5	21.8	0.2	15.8	10.6
	6C-4	282.5	17.5	51.0	1.0	8.2	1.01
	6D-4	292.5	0.5	61.1	0.2	13.4	1.69
	6E-4	67.4	0.7	17.4	0.2	13.2	3.21

[a] HMX concentration reported as mg/kg plant dry weight, mean of duplicate extractions.
[b] HMX concentration reported as mg/kg plant fresh weight, mean of duplicate extractions.
n.d. Not dectected.
n.a. Not applicable.

Table 7. HMX in plant samples, Final Sampling Event.

Treatment	Sample Prefix	Fresh shoots		Dried leaves		Roots	
		HMX[a] (mg/kg)	HMX[b] (mg/kg)	HMX[a] (mg/kg)	HMX[b] (mg/kg)	HMX[a] (mg/kg)	HMX[b] (mg/kg)
2	2A-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2B-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2C-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2D-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2E-1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3	3A-1	227.8	47.7			traces[c]	traces[c]
	3A-2	157.7	37.7			traces[c]	traces[c]
	3A-3	259.7	52.3			traces[c]	traces[c]
	3B-1	61.5	11.4	193.5	103.2	20.2	8.16
	3B-2	61.8	15.0	216.0	119.0	20.0	7.60
	3B-3	61.1	16.8			14.9	2.16
	3C-1	108.8	20.9	763 .0	261.0	31.3	7.46
	3C-2	85.4	24.4	677.2	204.0	34.1	5.12
	3C-3	107.2	22.0			39.1	9.44
	3D-1	195.6	43.6	267.2	227.0	19.6	11.2
	3D-2	739.6	215.0			11.2	4.20
	3D-3	1068	402.0	489.6	407.0	11.4	5.64
	3E-1	388.8	79.7	451.0	313.5	17.8	5.88
	3E-2	499.2	69.2	797.0	522.1	18.9	6.14
	3E-3	566.0	219.1			9.96	3.68
6	6A-1	301.8	80.0			traces[c]	traces[c]
	6A-2	349.8	97.7			traces[c]	traces[c]
	6A-3	243.2	67.9			traces[c]	traces[c]
	6B-1	69.4	14.8			19.0	3.26
	6B-2	29.6	6.52	162.5	51.1	21.4	5.78
	6B-3	27.4	4.30	197.0	52.2	16.2	1.90
	6C-1	106.8	48.6	768.0	566.2	17.5	10.6
	6C-2	219.6	48.8	658.0	506.6	14.5	12.6
	6C-3	177.2	45.9			24.1	13.6
	6D-1	612.4	184.2	533.8	322.8	6.80	1.16
	6D-2	369.4	96.1	322.8	276.0	53.1	17.6
	6D-3	272.3	73.5			48.8	8.64
	6E-1	101.3	31.4	212.8	135.6	11.7	4.64
	6E-2	476.8	121.7	305.4	212.4	24.9	6.14
	6E-3	205.4	.59.1			11.5	3.68

[a] HMX concentration reported as mg/kg plant dry weight.
[b] HMX concentration reported as mg/kg plant fresh weight.
[c] Majeur interference masking HMX, but traces identified by LC-MS.
n.d. Not detected.

LIST OF FIGURES

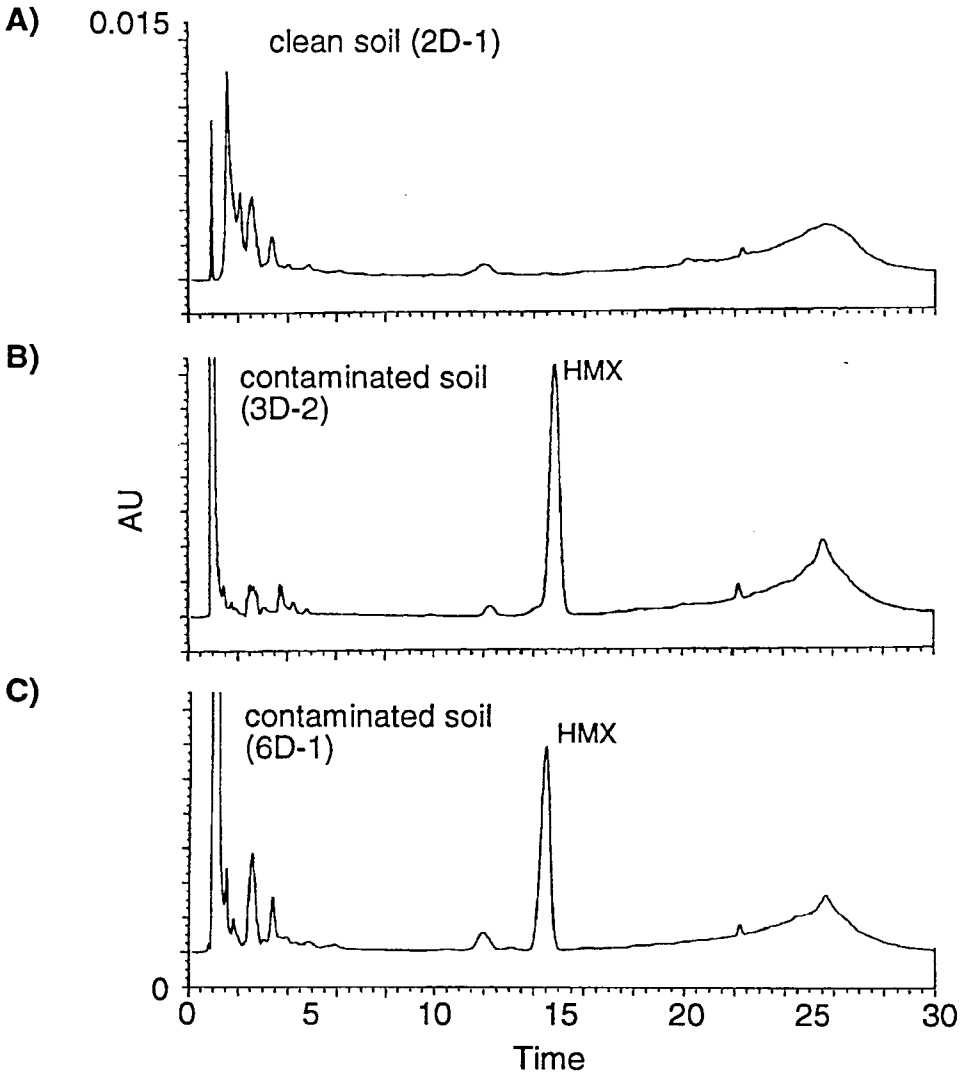

Figure 1: HPLC-UV chromatograms (254 nm) for wheat tissue extracts; (A) shoots grown in clean soil, (B) shoots grown in soil containing HMX (treatment 3), (C) shoots in soil containing HMX, iron and manure (treatment 6).

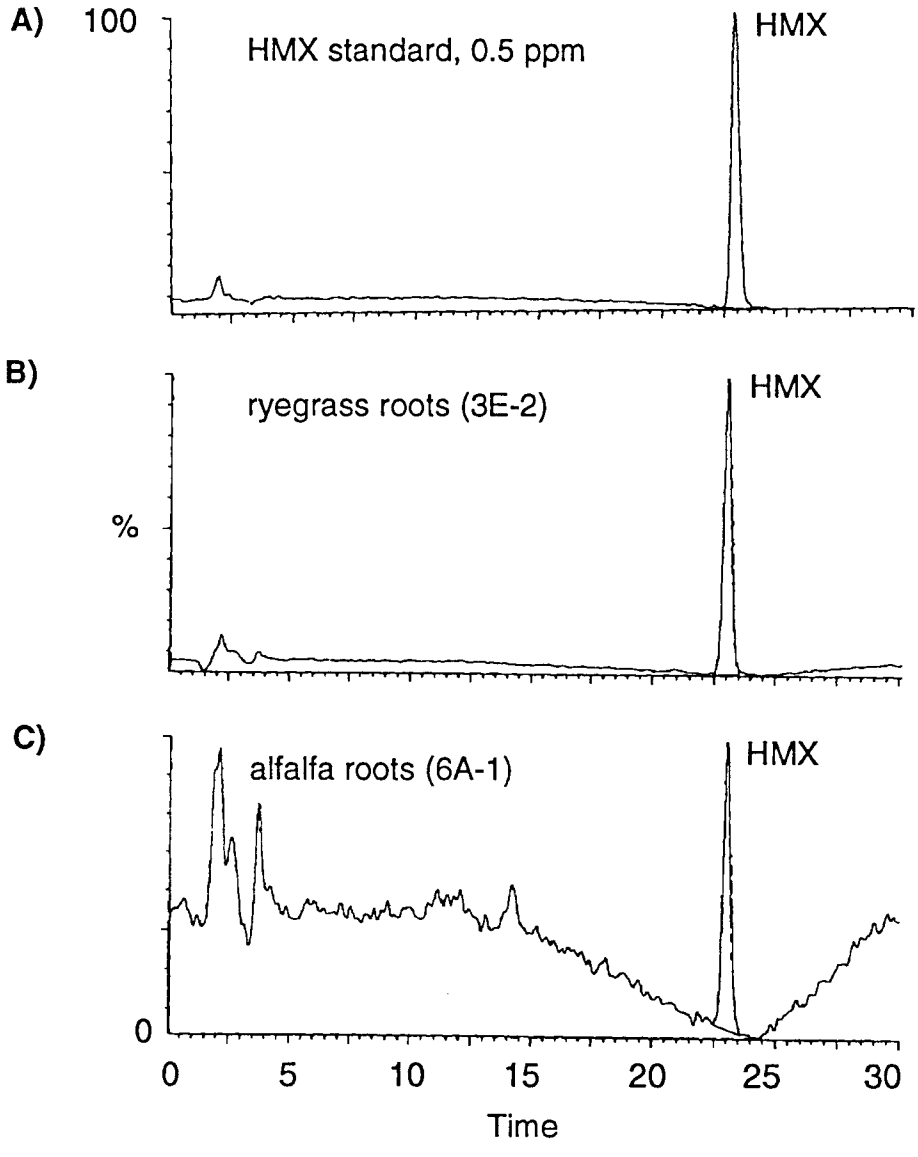

Figure 2: LC-MS characteristic mass ion extractions for HMX in root tissues; (A) 0.5 ppm HMX standard solution in acetonitrile, (B) ryegrass root extract (treatment 3), (C) alfalfa root extract (treatment 6).

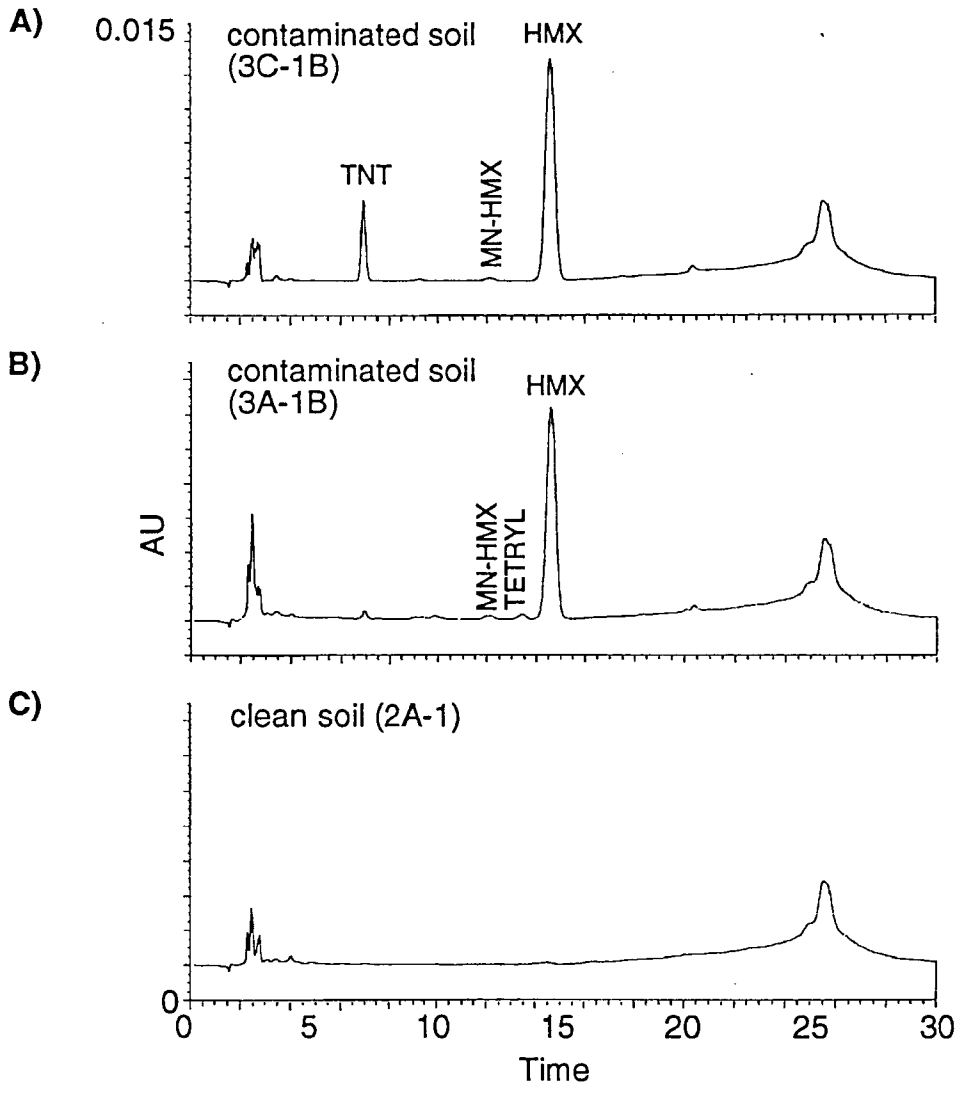

Figure 3: HPLC-UV chromatograms (254 nm) for soil extracts; (A) HMX contaminated soil treated with canola (treatment 3), (B) HMX contaminated soil treated with alfalfa (treatment 3), (C) clean soil (treatment 2). Note small quantities of TNT, Tetryl and MN-HMX in HMX contaminated soil.

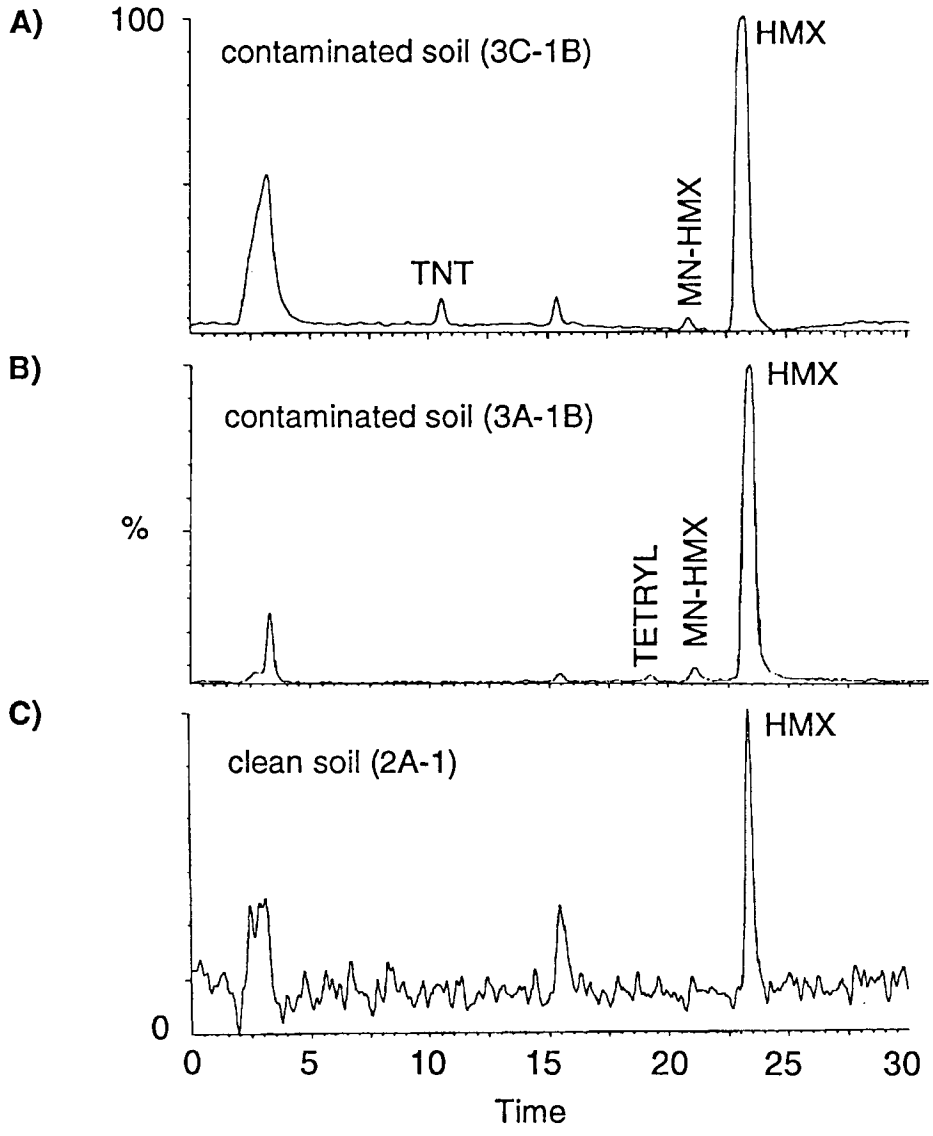

Figure 4: LC-MS characteristic mass ion extractions for TNT, Tetryl, 1-nitroso-3,5,7-trinitro-1,3,5,7-tetrazacylooctane (MN-HMX) and HMX in soil extracts; (A) HMX contaminated soil treated with canola (treatment 3), (B) HMX contaminated soil treated with alfalfa (treatment 3), (C) clean soil (treatment 2).

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